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FIELD TESTS OF *BACILLUS THURINGIENSIS* AND AERIAL APPLICATION STRATEGIES ON WESTERN MOUNTAINOUS TERRAIN

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usda douglas-fir tussock moth research & development program

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FIELD TESTS OF BACILLUS THURINGIENSIS *AND AERIAL APPLICATION STRATEGIES ON WESTERN MOUNTAINOUS TERRAIN*

Reference Abstract

Thompson, C. G., John Neisess, and Harold O. Batzer. 1977. Field tests of *Bacillus thuringiensis* and aerial application strategies on western mountainous terrain. USDA For. Serv. Res. Pap. PNW-230, 12 p., illus. Pacific Northwest Forest and Range Experiment Station, Portland, Oregon.

Aerial applications of *Bacillus thuringiensis* products were used to compare five formulations, three rates of application, and two spray droplet spectra. A formulation including a sunscreen was superior to the others tested, and the coarser spray atomization provided better spray coverage and residual activity.

KEYWORDS: Pesticide preparations, bioassay, virus (-forest pest control, *Bacillus thuringiensis*, Douglas-fir tussock moth, *Orgyia pseudotsugata*, western spruce budworm, *Choristoneura occidentalis*.

RESEARCH SUMMARY

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The effectiveness of aerial applications of five tank mixes of Dipel WP® and four Thuricide® treatments applied with different droplet spectra and application rates were evaluated against *Choristoneura occidentalis* Freeman and *Orgyia pseudotsugata* (McDunnough). Of the various tank mixes, the formulation of 25% Sorbo® and 0.5 lb/gal Shade® provided the best foliage protection and protected the viable *Bacillus thuringiensis* (Berliner) spores and prevented loss of insecticidal activity for at least 3 days. The coarser atomizations (> 300 µm vmd) produced superior spray coverage; the residual insecticidal activity lasted for 3 days posttreatment. The fine atomization (about 150 µm) started to degrade immediately.

Contents

Introduction

Commercial preparations of *Bacillus thuringiensis* Berliner *(B.t.)* are available from several manufacturers. These microbial insecticides have been very effective against some insect pests of agricultural crops where applications by ground equipment or by low-flying aircraft insure thorough coverage of the crop. Aerial applications of the same formulations and products against forest insects in mountainous terrain have often given erratic results (for example, Maksymiuk et al.,[1/] Maksymiuk and Neisess 1975, Stelzer et al. 1975, Stelzer and Neisess,[2/] Angus and Luthy 1971). The tests reported here were an effort to determine spray formulation and application characteristics needed for reliable forest defoliator control. Field populations of the western spruce budworm, *Choristoneura occidentalis* Freeman, and laboratory cultures of the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough), were used to measure insecticidal efficacy.

The variables tested were: application rate, droplet size, and effects of various spray adjuvants. Tests of application rate and droplet size were 0.5, 1.0, and 2.0 gallons per acre (4.67, 9.35, and 18.70 liters per hectare) and attempted droplet sizes of 150- and 300-um volume median diameters (vmd).[3/] Actual droplet size varied with spray formulation and atmospheric conditions. Dipel WP® was used for the spray adjuvant studies because it is a relatively simple product. Thuricide® was used for the studies on application rates and droplet sizes because it can be applied in lower volumes than the Dipel WP.

Materials and Methods

Dipel WP (16×10^3 international units/mg), a commercial wettable powder formulation of *B. thuringiensis*, was applied in five different tank mixes at the rate of 1 lb Dipel/2 gal per acre, which is equivalent to 7.264 billion international units of potency per acre (BIU/acre). Tank mixes are listed in table 1.

To evaluate the application parameters--droplet size and application rate--four Thuricide mixtures were applied as follows: 8 BIU of Thuricide 16B/gal per acre (8 BIU/9.35 liters per hectare) with coarse spray atomization; 8 BIU of Thuricide 16B/gal per acre (8 BIU/9.35 liters per hectare) with fine atomization; 8 BIU of Thuricide 24B/0.5 gal per acre (8 BIU/4.675 liters per hectare) with fine atomization; 8 BIU of Thuricide 32B/2 gal per acre (8 BIU/18.7 liters per hectare) with fine atomization. The 2-gal/acre (18.7 liters/acre) treatment was formulated in a 25% Sorbo® solution in water. The other treatments were formulated in water (table 1).

[1/] Maksymiuk, Bohdan, P. A. Boving, R. D. Orchard, and R. G. Winterfeld. 1968. Forest tests to develop an operational method to control the Douglas-fir tussock moth with a polyhedrosis virus. Progress report, USDA For. Serv., Pac. Northwest For. and Range Exp. Stn., Forestry Sciences Laboratory, Corvallis, Oregon, and Agric. Eng. Res. Division, Forest Grove, Oregon. 33 p.

[2/] M. J. Stelzer and J. A. Neisess. 1974. 1975 Field experiments with *Bacillus thuringiensis* to control Douglas-fir tussock moth in Idaho. Study No. 3. Douglas-fir tussock moth research and pilot test program, season of 1974. Interim report, USDA For. Serv., Pac. Northwest For. and Range Exp. Stn. 9 p.

[3/] Volume median diameter (vmd) is the drop diameter satisfying the requirement that half of the volume of spray is in drops smaller and half in drops larger than the vmd.

Table 1 --*Summary of treatments and application parameters, Ellensburg, Washington, 1975*

Test parameter	Active ingredient	Tank mix	Application rate	Proposed atomization
	BIU[a]/acre		Gal/acre[b]	Micrometers
Formulations	7.264 BIU Dipel WP	H_2O + 0.125% BioFilm[c]	2	300
	7.264 BIU Dipel WP	H_2O + 25% CIB molasses	2	300
	7.264 BIU Dipel WP	H_2O + 25% Sorbo[d]	2	300
	7.264 BIU Dipel WP	H_2O + 25% Sorbo + 0.5 lb/gal shade[e]	2	300
	7.264 BIU Dipel WP	H_2O + 25% Sorbo + 0.1% AL 411F sticker[f]	2	300
Application parameters	8 BIU Thuricide 16B	50:50 with H_2O	1	300
	8 BIU Thuricide 16B	50:50 with H_2O	1	150
	8 BIU Thuricide 24B	67:33 with H_2O	0.5	150
	8 BIU Thuricide 32B	25% Sorbo + 50% H_2O + 25% Thuricide 32B	2	150
	Untreated control			

[a] BIU - billion (10^9) international units.

[b] One gallon per acre equals 9.35 liters per hectare.

[c] Colloidal Products Corp., Petaluma, Calif.

[d] A 70% Sorbitol solution which is a polyhydric alcohol derived from D-glucose, ICI United States, Inc., Wilmington, Del.

[e] Sandoz, Inc., Homestead, Fla., 0.5 lb/gal = 0.0599 kg/l.

[f] ICI United States, Inc., Wilmington, Del.

Rhodamine B Extra S[4] was added to each tank mix at the rate of 3.785 g/gal (1 g/liter) for spray-deposit assessment. Tank samples were collected for each replicate of every treatment for the determination of dye and *B. thuringiensis* concentrations.

EXPERIMENTAL LAYOUT

The study was conducted on thirty 15-acre (6.075 hectares) plots that were established in stands of Douglas-fir, *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco, and grand fir, *Abies grandis* (Dougl.) Lindl., infested with spruce budworm in the Wenatchee National Forest north of Ellensburg, Washington. Near the center of each plot, 15 trees were designated for population, residue, and spray-deposit sampling. Sample trees were Douglas-fir or grand fir, 30 to 50 feet (9.144 to 15.240 meters) tall, open-grown, and not shielded by larger trees. Because all plots were within one continuous area, the same checks were used for each part of the field experiment. The treatments were assigned to the plots at random to give a completely random design of three replicates of each treatment.

A Hiller 12-E helicopter equipped with either conventional spray boom and flat fan nozzles or a Bals Turbair Spinning Disk spray system was used to apply all materials. The boom and nozzles, fitted with Spraying Systems (Wheaton, Ill.) T8002 flat fan tips, were used to apply the coarse atomization treatments. The nozzles were oriented forward and down 45° to the line of flight of the helicopter. The ambient boom pressure was 60/lb

[4] General Aniline Film Corp., Portland, Oregon.

per in^2 (4.218 kg/cm). The spinners were calibrated to spin at 9,361 revolutions per minute (rpm) without any liquid flowing. The spinning speed was reduced to about 9,000 rpm when the spray was applied. The helicopter sprayed at 45 mph (72.42 km/h) at about 50 ft (15.240 m) above the trees. Swath spacings ranged between 50 and 100 ft (15.240 and 30.48 m) depending on the application rate and desired flow rate of each treatment.

Plots were sprayed when 70% of the budworm population had reached the fourth larval instar.

EVALUATION

Measurements of the density of western spruce budworm populations were made 1 to 2 days before treatment and 1, 2, 3, 5, 7, and 14 days after treatment and at pupation.

At each sampling interval, four 15-inch (38.1-cm) branches with a minimum of 50 shoots were collected from the midcrown of each sample tree. In the field laboratory, the current year's shoots were counted, as well as the number of budworm larvae per branch. Population estimates were expressed as budworms per 100 new shoots. At each sampling interval, up to 10 larvae were collected from foliage samples from each sample tree and placed in individual disposable petri dishes containing an artifical diet (McMorran 1965). These dishes were held in the laboratory at 25°C and 40-50% relative humidity (RH) for 14 days; then the dead larvae were examined microscopically to diagnose the cause of death (that is, bacterial infection, parasitism, or unknown).

Spray deposit was sampled on the ground with aluminum plates and white Kromekote® cards. Volume median diameter (vmd) of droplets and densities of droplets on the Kromekote cards were measured with a Quantimet® 720 particle analyzer. Spray deposits were removed from the aluminum plates with 10 ml of distilled water, and amount of spray deposit was determined by fluorometric analysis (Maksymiuk et al. 1975). Deposit values were expressed as gallons per acre. Spray residues were determined from the same midcrown branches used for spruce budworm counts. Foliage was collected from every sample tree immediately postspray (about 30 minutes), and at 1, 2, 3, 5, and 7 days postspray. Samples were divided into two subsamples, one for fluorometric analysis and one for bioassay.

After the foliage samples had been dried, 5-g and 200-needle subsamples were selected from each foliage sample. Spray residues were removed from the 5-g samples by washing with 50 ml of distilled water. Spray deposit, in terms of concentration of fluorescent dye, was measured with a Model 430 Turner Spectrofluorometer. The volume of spray per gram of foliage was determined from the amount of dye removed from the needles and the dye concentrations in the tank samples. The 200-needle samples were weighed, and the number of spray droplets were counted using a dissecting microscope with an ultraviolet light for illumination of the fluorescent deposit. The counts were expressed in terms of droplets per 100 needles and percentage of needles that had at least one spray droplet.

The foliage samples designated for bioassay were dried, and 5 g of needles from each tree were placed in 4-oz (29.57-ml) plastic cups. Fifty ml of distilled water was pipeted into each cup, and the cup was agitated for about 2 min on a Maxi Mix® model M-16715 stirrer. One drop (0.025 ml) of each wash suspension was spread on the surface of artificial medium in a 1/2- by 2-inch (1.27- by 5.08-cm) clear polystyrene vial. Individual neonatal Douglas-fir tussock moth larvae from our laboratory colony were placed on the treated diet, and the vials were sealed with Parafilm®. Mortalities

were recorded after the larvae were held at 26°C and 45% RH for 14 days. Three replicates (10 larvae/replicate, 1 foliage sample/rep per treatment) were tested for each treatment and sample interval. Control groups of 30 larvae, exposed to untreated diet, were set up for each 150 sample vials.

Three-ml portions of the wash solution from each of the immediate postspray samples were placed in 1/2- by 2-in (1.27- by 5.08-cm) vials and pasteurized for 15 minutes in an 80°C water bath. The wash suspensions were plated on nutrient agar by standard bacteriological techniques. Developing colonies were spot checked for *B.t.* endospores. Viable spore counts were expressed as spores per gram of foliage.

Defoliation estimates were made on the final foliage samples based on the average new foliage loss on each shoot, by a method similar to that described by Heller and Schmiege (1962).

ANALYSIS

Spruce budworm survival, initial infection rate, Douglas-fir tussock moth mortality from the bioassay of foliage samples collected immediately postspray, viable *B.t.* spore counts, and deposit data were subjected to analysis of variance. Data pertaining to the two studies, formulation and application variables, and for different sampling intervals, were analyzed separately. Defoliation data was subjected to analysis of covariance using the prespray density of spruce budworms as the covariate. In each analysis of variance or covariance, the sum of squares among treatments was partitioned into sets of orthogonal single degree-of-freedom comparisons to test differences in treatment parameters. Data from bioassay of Douglas-fir tussock moth and rate of spruce budworm infection for all the sampling intervals were analyzed by analysis of variance with time (sampling intervals) as a split plot factor.

Results and Discussion

FORMULATIONS

Although the five different Dipel tank mixes were applied under the same application parameters, different droplet size spectra existed with the Dipel-BioFilm® tank mix having the smallest vmd (table 2). Table 3 summarizes the single degree-of-freedom contrasts that evaluate and compare the various spray adjuvants. The addition of the sticker to the Sorbo mixture increased the viscosity of the solution enough to increase the atomization significantly. Although the vmd for the BioFilm tank mix was significantly ($p \leq 0.01$) lower than the molasses treatment, this finer atomization did not result in significantly higher droplet densities or spray volumes either at the ground or midcrown. Deposit results for BioFilm and molasses are consistent with those found by Stelzer et al. (1975), who attributed the lower deposit of the BioFilm tank mix to the higher evaporation rate. Besides droplet size, atomization-dependent deposit variables, such as drops per needle, varied for some treatments (table 2).

Fluorometric analysis of the Sorbo + Shade® treatment gave erroneous results for gallon per acre and microliters of spray per gram of foliage. We found in later laboratory experiments that fluorescent intensity of the Sorbo + Shade + dye solution declined over time. Tank samples were stored about 3 to 4 months before analysis, so the analyzed dye concentrations were 4 to 5 times lower than the concentrations of dye actually added to the tank mixes. Because the dried deposits did not fade (on the plates and foliage), the deposit values analyzed 4 to 5 times higher than the real values.

Table 2 --*Deposit data for 1 lb Dipel WP/2 gal per acre (7.26 BIU/acre) mixed with various spray adjuvants, Ellensburg, Washington*

Formulation	Vmd	Droplets/cm^2	Droplets/needle	Droplets with needles	Amount recovered	Spray/ foliage
	Micrometers	- - - - - - Number - - - - - -		Percent	Gal/acre	µl/g
0.125% BioFilm	192	13.0	1.614	51.6	0.642	0.074
25% CIB molasses	355	16.0	1.130	48.3	0.857	0.223
25% Sorbo	274	7.7	0.421	26.5	0.294	0.128
25% Sorbo + 0.5 lb Shade/gal	289	11.7	0.282	12.5	3.209[a/]	0.976[a/]
25% Sorbo + AL 411F Sticker	363	13.0	0.968	47.2	0.542	0.115

[a/] Fluorometric analysis was inhibited by Shade adjuvant. Table values are 4-5 times higher than actual volumes.

Table 3 --*Summary of F values for single degree of freedom comparisons of the effect of Dipel WP formulations mixed with various spray adjuvants on deposit parameters*

Contrast	Vmd	Droplets/cm^2	Droplets/needle	Needles with droplets	Amount recovered	Spray/ foliage
Sorbo vs. non-Sorbo formulations	4.330 NS	1.067 NS	5.898 *	7.128 *	--	--
Molasses vs. BioFilm	39.215 **	0.289 NS	0.869 NS	0.070 NS	0.400 NS	3.164 *
Sorbo vs. Sorbo with additives	5.309 *	0.932 NS	0.206 NS	0.101 NS	--	--
Sorbo + Sticker vs. Sorbo + Shade	8.043 *	0.057 NS	1.743 NS	7.918 *	--	--

* = significant at 0.05 level, 1 and 10 df.

** = significant at 0.01 level, 1 and 10 df.

NS = nonsignificant.

Survival ratios of western spruce budworms did not differ for any of the treatments until the 14-day sample. Then the mean survival ratio for all Dipel tank mixes was significantly lower than the control (table 4). Apparently the 60% increase in initial infection rate (table 4) achieved with the molasses treatment compared to the BioFilm treatment was not great enough to cause differences in the survival ratio measured in the field. No other differences in the infection rate were significant (table 5). The survival data (table 4) corroborate the defoliation data where all the Dipel mixtures provided significantly ($p \leq 0.05$) better foliage protection than the control. Although the survival ratio for the Sorbo + Shade treatment was not significantly different from the other Sorbo treatments, the effect of the reduced survival rate for this treatment was observed in the defoliation data. Foliage protection provided by the Sorbo + Shade treatment was significantly greater than the protection

Table 4 --*Biological responses to 0.5 lb Dipel WP/gal mixed with various spray adjuvants, Ellensburg, Washington*

Treatments: Dipel WP +	Prespray budworm population (larvae/ 100 shoots)	Spruce budworm survival ratio			Defoliation rate	Infection rate in budworm	0-day viable *B.t.* spore/g foliage x 10^3 [a/]	0-day DFTM mortality
		5-day	7-day	14-day				
						Percent		Percent
0.125% BioFilm	18.0	1.203	1.203	0.873	0.556	25.3	50.7	53.9
25% molasses	25.3	1.020	0.987	0.693	0.490	40.7	79.3	77.0
25% Sorbo	24.7	0.917	0.773	0.723	0.586	35.3	96.0	56.1
25% Sorbo + 0.5 lb Shade/gal	29.6	0.897	0.747	0.390	0.394	47.0	206.0	58.9
25% Sorbo + AL 411F Sticker	30.6	0.960	1.137	0.633	0.637	39.3	214.0	77.8
Untreated control	30.7	0.890	1.220	1.217	0.713	0.0	0.0	19.1

[a/] Samples collected 30 minutes postspray.

Table 5 --*Summary of F values for individual degree of freedom comparisons of means in analysis of variance of spruce budworm survival and infection data, Douglas-fir tussock moth mortalities, viable spore counts, and defoliation data*

Contrast 1 and 12 df	F values						
	Spruce budworm survival ratio			Defoliation rate	Infection rate	Viable spores/ g foliage	0-day DFTM mortality
	5-day	7-day	14-day				
All treatments vs. control	0.853 NS	2.329 NS	6.544 *	5.110 *	--	--	44.358 **
Sorbo vs. non-Sorbo. (1+2 vs. 3+4+5)	3.604 NS	2.341 NS	1.242 NS	0.056 NS	3.773 NS	7.089 *	0.205 NS
Molasses vs. BioFilm	1.440 NS	1.044 NS	0.415 NS	0.397 NS	6.475 *	0.211 NS	7.827 *
Sorbo vs. Sorbo with additives	0.008 NS	0.840 NS	0.764 NS	0.628 NS	2.253 NS	4.467 *	3.606 NS
Sorbo + Sticker vs. Sorbo + Shade	0.172 NS	3.381 NS	0.758 NS	5.760 *	1.619 NS	0.017 NS	4.982 *

* = significant at 0.05 level, 1 and 10 df.

** = significant at 0.01 level, 1 and 10 df.

NS = nonsignificant.

provided by the Sorbo + sticker.

All of the Dipel mixtures had significantly higher initial Douglas-fir tussock moth bioassay mortalities than the control (tables 4 and 5). The formulation with molasses gave significantly higher ($p \leq 0.05$) mortalities than the one with BioFilm. Likewise, the addition of the sticker to the Sorbo mixture significantly ($p \leq 0.05$) increased the bioassay mortalities. The increased mortality of the Sorbo + sticker treatment can be partially explained by the increased deposit (tables 2 and 3), especially the percentage of needles with spray drops. Because the deposits for the molasses treatments were not significantly higher than the BioFilm treatment (tables 2 and 3), some other

factor must explain the higher bioassay mortalities. Molasses could be acting as a gustatory stimulant. Yendol et al. (1975) reported such a stimulant with gypsy moth, *Lymantria dispar* (L.), fed commercial *B.t.* preparations. The addition of Shade and sticker to the Sorbo mixture apparently protected the viable spores from degradation, even while the spray droplets were airborne (table 4), because the number of viable spores for these two treatments were significantly higher (table 5) than the Sorbo-only treatment. If the adjuvants protected the spray droplets only after they were deposited on the foliage, the viable spore counts from the initial postspray sample should have been about equal, and significant differences in spore counts would only show up in the subsequent daily residue samples.

Analysis of variance of the residual activity data (Douglas-fir tussock moth bioassay and spruce budworm infection rate for the various sampling intervals) where time was a factor showed significant treatment by time interactions ($F = 1.74$ with 36 and 80 df and $F = 6.492$ with 40 and 90 df, respectively). This significant interaction indicated that various treatments responded differently over time. Figure 1 shows a plot of bioassay mortality against time for two such treatments, Sorbo and Sorbo + Shade. The addition of Shade obviously affected the residual activity of the *B.t.* The activity of the Sorbo treatment steadily declined from the initial postspray sample, but the activity of the Sorbo + Shade treatment remained at virtually 100% until after the 3-day sample. Plots of the spruce budworm infection data showed the same trend, except the activity of the Sorbo + Shade treatment

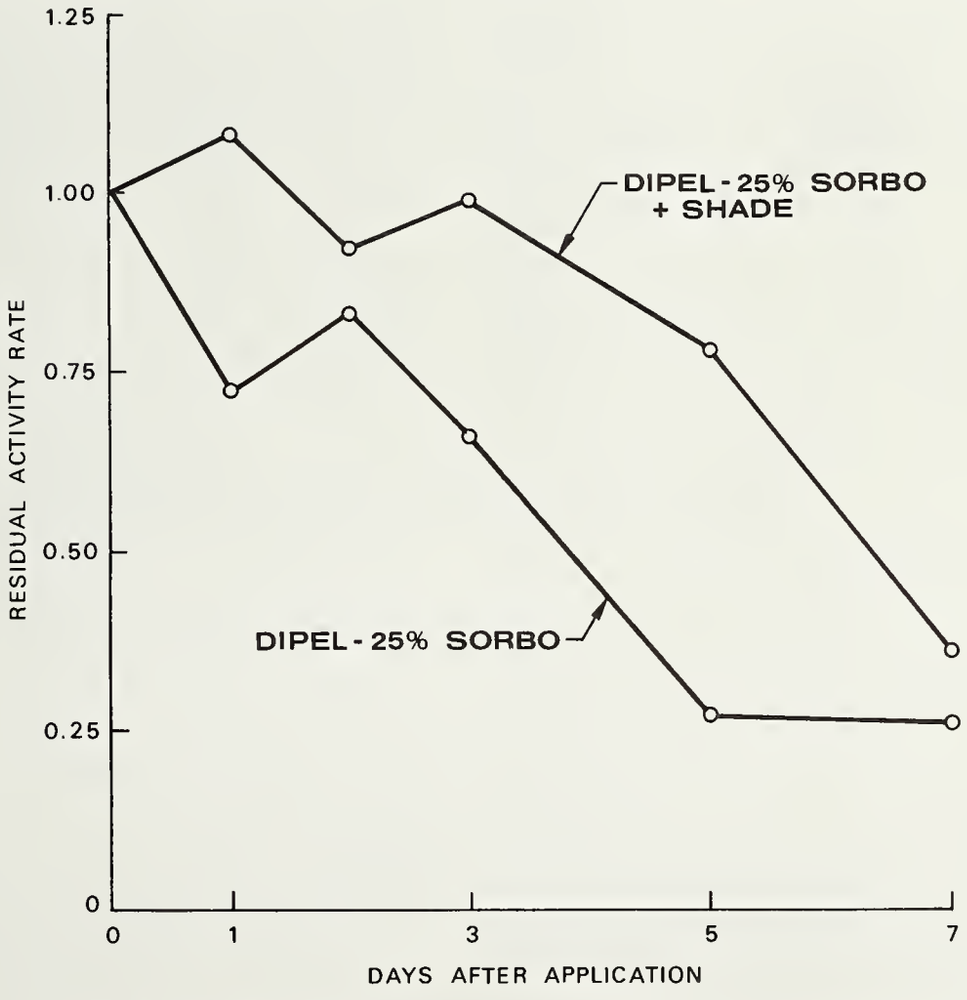

Figure 1.--Residual activity of Bacillus thuringiensis *formulated with and without a sunscreen.*

remained at 100% for about 5 days. These differences in residual activity result from addition of Shade to the mixture, because initial mortality and deposit values were equivalent for these two treatments.

DROPLET SIZE AND APPLICATION RATE

Although the fine droplet spectra (164-µm) resulted in significantly more droplets per cm^2, the coarse atomization (411-µm) allowed significantly greater volumes of spray to reach the midcrown and ground levels (tables 6 and 7). Although enough variations existed in the values for droplets per needle and percentage of needles with droplets that observed differences could not be concluded as real differences, coverage for the coarser atomization of this experiment would have to be judged superior to that of the fine droplet spectra. This is so because coverage is a compromise between volume and number (droplets/area). At first glance, the practical significance of these differences in deposit are questionable because tables 7 and 8 show that defoliation was the only biological response with a significant difference ($p \leq 0.05$). We found no significant differences between the two droplet sizes in spruce budworm survival, initial mortality in the Douglas-fir tussock moth bioassay, viable spore counts, or rate of spruce budworm infection. This was because of the biological variability encountered in these tests. Analysis of variance of the residual activity data, when time

Table 6 --*Deposit data resulting from changes in application parameters-- droplet size and application rate*

Treatment	Application rate	Vmd	Droplets/cm^2	Droplets/needle	Needles with droplets	Amount recovered	Spray/foliage
	(Gal/acre)	(Micrometers)	---- Number ----		Percent	Gal/acre	µl/g
Thuricide 16B	1.0	411	7.3	0.925	43.1	1.177	0.316
Thuricide 16B	1.0	164	16.7	0.422	23.8	0.144	0.018
Thuricide 24B	0.5	172	12.3	0.123	9.6	0.085	0.042
Thuricide 32B	2.0	220	15.7	0.733	32.9	0.229	0.070

Table 7 --*Summary of F values for single degree of freedom comparisons of means in analysis of variance of spray deposit data for changes in droplet size and application rate of four Thuricide treatments*

Contrast	Vmd	Droplets/cm^2	Droplets/needle	Needles with droplets	Amount recovered	Spray/foliage
Fine drops vs. coarse (164 vs. 411 vmd)	68.546 **	7.649 *	2.608 NS	3.725 NS	519.618 **	20.211 **
1 gal/acre vs. ½ + 2 gal	18.763 **	0.702 NS	1.244 NS	2.967 NS	246.728 **	5.591 *
½ gal/acre vs. 2 gal/acre	2.546 NS	0.976 NS	3.835 NS	5.426 *	10.151 *	0.174 NS

* = significant at 0.05 level, 1 and 10 df.
** = significant at 0.01 level, 1 and 10 df.
NS = nonsignificant.

Table 8 --*Biological response variables for application parameters, droplet size, and application rate*

Treatment and parameter	Prespray budworm population (larvae/ 100 shoots)	Spruce budworm survival ratio			Defoliation rate	0-day DFTM mortality	Viable spore/ g of foliage x 10^3	Infection rate in budworm
		5-day	7-day	14-day				
						Percent		Percent
Thuricide 16B large drops (411µm)	27.0	0.923	1.063	0.547	0.576	46.4	335.0	42.0
Thuricide 16B small drops (164µm) 1 gal/acre	33.4	0.807	0.697	0.553	0.765	46.3	132.1	47.7
Thuricide 24B 0.5 gal/acre	25.6	0.987	0.710	0.443	0.480	68.7	268.2	29.0
Thuricide 32B 2 gal/acre	31.3	0.583	0.497	0.387	0.626 NS	50.4	73.3	51.0
Untreated control	30.7	0.890	1.220	1.217	0.762 NS	17.0	0.0	0.0

(sample interval) was a factor, however, showed significant differences between treatments. Figure 2 illustrates how the bioassay mortalities varied over time for the two droplet sizes. The initial residual activity of the coarse droplet spectra lasted for 3 days postspray, but the activity of the fine droplets started to degrade immediately. Because the volume of spray in the coarse droplets was significantly greater (tables 6 and 7) than the fine droplets, this result was not unexpected.

Application at 2 gal/acre (18.70 liter/ha) consistently provided the best deposit (table 6), with values for percentage of needles with droplets and gallons per acre significantly higher than at 0.5 gal/acre (4.675 liter/ha). The inconsistent data on droplets per square centimeter for the various treatments may have been because the cards were too close to surrounding trees, which could have screened some of the spray. The only biological responses that paralleled the deposit results were the infection rate (table 8) and the 5-day spruce budworm survival, and these responses did not consistently correlate with the defoliation data. Although the 2-gal/acre (18.70-liter/ha) treatment resulted in a significantly higher initial rate of infection, defoliation resulting from the 2-gal/acre (18.70-liter/ha) treatment appears (difference was not significant, table 9) to be greater than with 0.5 gal/acre (4.675 liter/ha) (table 8). This inconsistency can partly be explained by the difference in density of spruce budworm larvae before spraying. Although analysis of covariance was used to adjust the defoliation rates according to initial larval densities, no prespray defoliation measurements were recorded. The applications were timed against fourth-instar larvae, so considerable defoliation had occurred before treatment--especially in plots with the higher densities--which our analysis could not properly evaluate. Stelzer et al. (1977) observed that with 1- and 2-gal/acre (9.35- and 18.7-liter/ha) applications of baculovirus against Douglas-fir tussock moth, all deposit values from the 2-gal/acre (18.7-liter/ha) application rates were

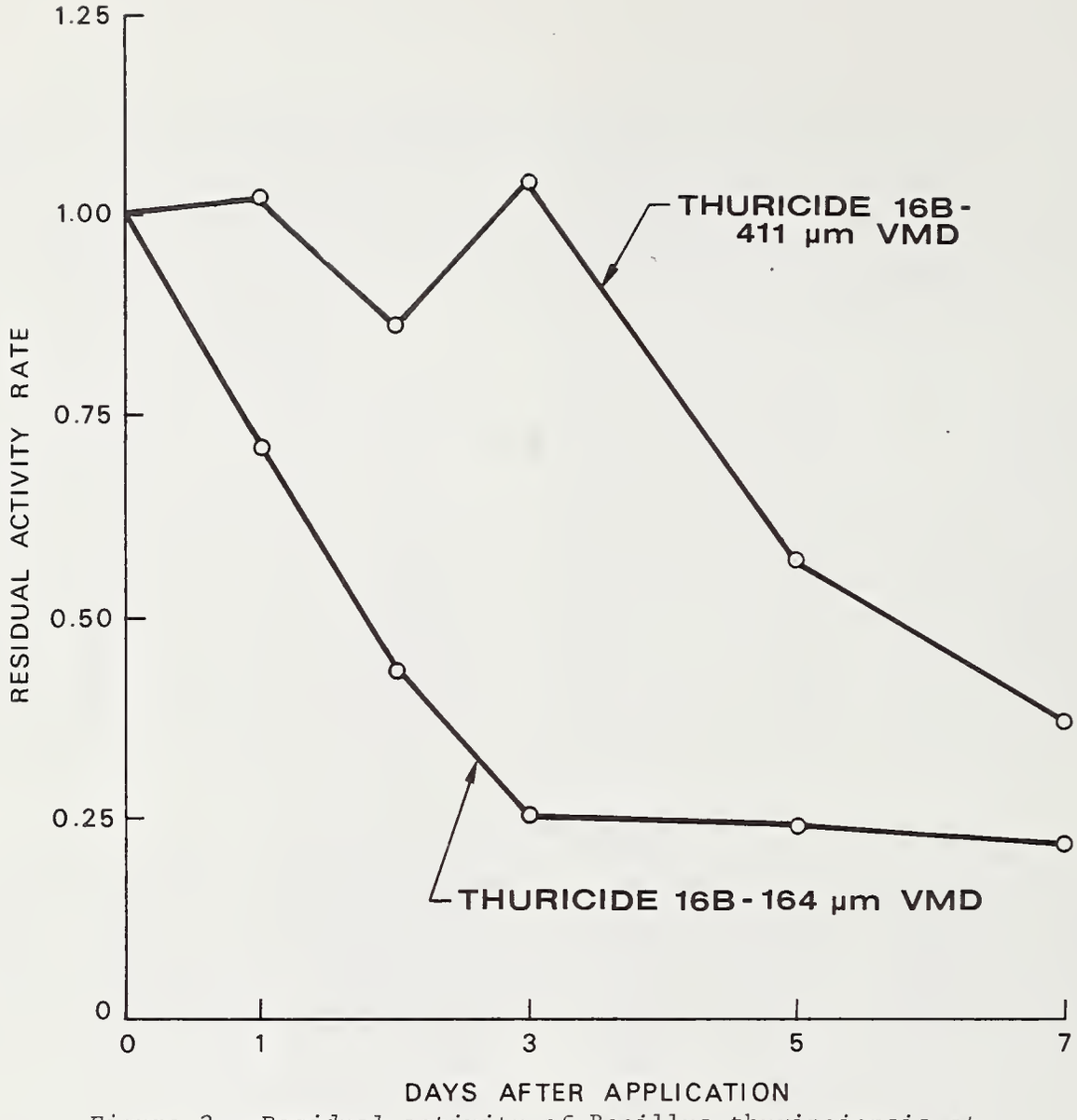

Figure 2.--Residual activity of Bacillus thuringiensis *at two droplet ranges.*

Table 9 --*Summary of F values for orthogonal comparisons of means from the analysis of variance of biological response variables for the application parameters droplet size and application volume*

Contrast	Spruce budworm survival			Defoliation rate	0-day DFTM mortality	Viable spores/ g of foliage	Infection rate
	5-day	7-day	14-day				
All treatments vs. control	0.238 NS	11.207 **	14.422 **	6.003 *	15.178 **	--	--
Small drop vs. large	0.480 NS	4.116 NS	0.001 NS	5.6593 *	0.000 NS	2.507 NS	1.151 NS
1 gal/acre vs. ½+2 gal/acre	0.451 NS	4.686 NS	0.610 NS	4.5927 NS	2.565 NS	0.535 NS	1.675 NS
½ gal/acre vs. 2 gal/acre	5.733 *	1.393 NS	0.054 NS	3.389 NS	2.459 NS	1.918 NS	17.355 **

* = significant at 0.05 level, 1 and 10 df.
** = significant at 0.01 level, 1 and 10 df.
NS = nonsignificant.

about double the 1-gal/acre (9.35-liter/ha) values, but the biological responses were equal. The reason our experiment did not provide such clearcut differences in deposit value might be the small droplet size--around 150-µm. The droplet sizes used in the virus experiment were 200 to 300-µm. Our variable data seem to indicate that the droplet size may have been too fine to yield uniform deposition, which would explain the inconsistent results in our response variables. Also budworms are more numerous in the upper portion of the tree crown, and defoliation in the upper crown may force this portion of the population down into the midcrown. As a result, populations in some test plots increased in our midcrown samples. The average budworm population for one treatment (1 gal/acre (9.35 liter/acre), small droplets) increased from 33 larvae/100 shoots prespray to 45 larvae/100 shoots 3 days after spraying. Because all population reduction figures were based on reduction from prespray counts, they did not always reflect actual conditions.

EFFECT OF *B. t.* TREATMENTS ON INSECT PARASITISM

In determining causes of mortality in laboratory rearings of budworm samples collected from each plot, we recorded budworm larvae killed by insect parasites. The percentage of parasitism in larvae not killed by *B.t.* in all treated plots was almost identical to that in larvae from the control plots (fig. 3). This finding supports the environmental compatibility of *B.t.* treatments with parasitic insect species. We did find that two of the major parasites observed in our studies (*Glypta fumiferanae* (Viereck) and an *Apantelei* sp.) could acquire lethal *B.t.* infections from their host budworm. Not uncommonly, we observed apparently fully developed parasite larvae emerging from their host only to die before they could pupate. These parasites demonstrated typical symptoms of advanced infection and were always packed with all stages of *B.t.* Almost always, the host budworm larvae remained alive until the parasite larvae had emerged. Few parasites emerged from hosts that had been dead (from *B.t.* infection) for any length of time. Thus, although the *B.t.* treatments appear to have had no effect on adult parasites or those immature parasites in uninfected hosts, these treatments did reduce the number of parasites surviving host infections--even where the host remained alive long enough for the parasite to mature sufficiently to emerge and, sometimes, even spin a cocoon.

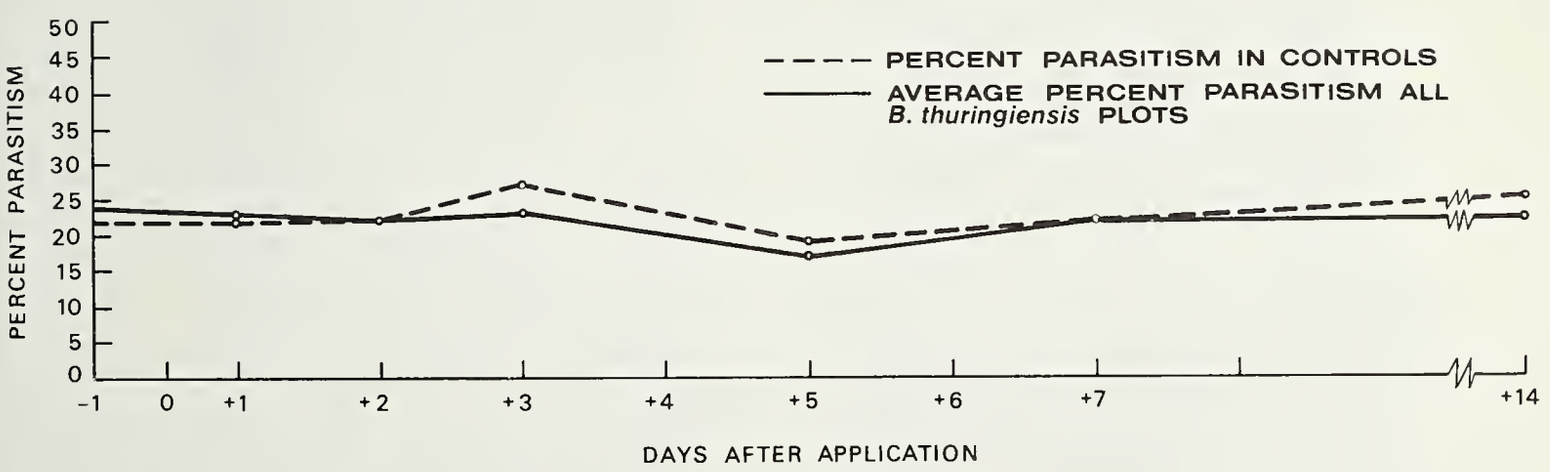

Figure 3.--Percent parasitism in budworm rearing samples not killed by B.t.

Conclusions

Although not all the test objectives were fully realized, the results justify these conclusions:

1. The addition of a sticker to the Sorbo mixture increased viscosity and spray droplet size.

2. The BioFilm mixture produced finer atomization, but this did not result in greater coverage.

3. All treatments significantly reduced budworm populations; but under the test conditions, none of the treatments provided satisfactory population regulation.

4. Foliage protection was greater with the Sorbo and Shade formulation than with the Sorbo and sticker.

5. The addition of Shade to the Sorbo formulation protected viable spores from degradation for 3 days. Insecticidal activity of spray deposit with the Shade formulation was extended to 5 days.

6. The coarser atomizations appeared to produce superior spray coverage. Residual activity of the coarse droplets lasted for 3 days postspray, but the fine droplets started to degrade immediately.

7. Insect parasitism was not affected in the budworm populations that escaped the *B.t.* treatments.

8. Two species of parasites readily acquired *B.t.* from infected host budworms.

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KEYWORDS: Pesticide preparations, bioassay, virus (-forest pest control, *Bacillus thuringiensis*, Douglas-fir tussock moth, *Orgyia pseudotsugata*, western spruce budworm, *Choristoneura occidentalis*.

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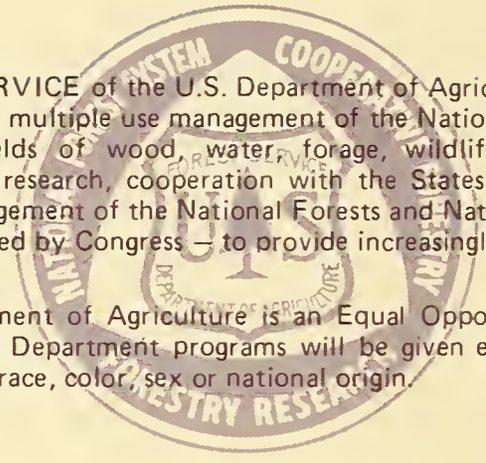

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